
Use of the DICE (Dual Integrase Cassette Exchange) System.

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Public Summary:

This paper gives detailed instructions to those wanting to use the dual integrase cassette exchange (DICE) method. The DICE method is useful for making precise insertions into a mammalian genome at a pre-determined location. Because the method is efficient, it is suitable for making a series of different insertions at the same location. To date, the DICE method has been most utilized in the context of human induced pluripotent stem cells. The method provides a much-needed genetic engineering tool for these iPSC and is being used by academic labs and companies to engineer iPSC to increase basic science understanding and to develop therapeutics. Therefore, this article will allow other users to benefit from the expertise the Calos lab developed with the DICE method.

Scientific Abstract:

When constructing transgenic cell lines via plasmid DNA integration, precise targeting to a desired genomic location is advantageous. It is also often advantageous to remove the bacterial backbone, since bacterial elements can lead to the epigenetic silencing of neighboring DNA. The least cumbersome method to remove the plasmid backbone is recombinase-mediated cassette exchange (RMCE). RMCE is accomplished by arranging recombinase sites in the genome and in a donor plasmid such that a recombinase can both integrate the donor plasmid and excise its bacterial backbone. When a single recombinase is used for RMCE, recombination between undesired pairings of the sites can lead to a significant number of unwanted cell lines. To reduce the frequency with which these side products occur, several variants of RMCE that increase desired outcomes have been developed. Nevertheless, an important feature lacking from these improved RMCE methods is that none have fully utilized the recombinases that have been demonstrated to be the most robust and stringent at performing genomic integration in plants and animals, i.e., the phiC31 and Bxb1 phage integrases. To address this need, we have developed an RMCE protocol using these two serine integrases that we call dual integrase cassette exchange (DICE). Our DICE system provides a means to achieve high-precision DNA integration at a desired location and is especially well suited for repeated recombination into the same locus. In this chapter, we provide our most current protocols for using DICE in feeder-free human-induced pluripotent stem cells.

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